Towards energy efficient and healthy buildings: an overview how (not) to get a *Legionella pneumophila* infection

Elisa Van Kenhove[1,*], Jelle Laverge[1], Arnold Janssens[1]

[1]Ghent University (UGent), Ghent, Belgium

[*] *Corresponding email: Elisa.VanKenhove@UGent.be*

SUMMARY
The total energy demand of energy efficient buildings raised some questions about the production of domestic hot water (DHW). One of the main reasons for the high energy demand is that DHW is produced, stored and distributed at temperatures above 55°C to mitigate the risk of infecting the DHW system with *Legionella pneumophila*. At these temperatures, *L. pneumophila* species growth is stopped and remaining species are effectively killed. Over the preceding two decades less research had been published on the progress in knowledge on the infection process and disinfection techniques. This paper offers an overview of the profitable conditions for <u>*Legionella pneumophila* growth</u> (lukewarm water between 20 and 45°C, stagnation, an acid environment and the presence of nutrients), <u>infection process</u> (profitable conditions, creation of aerosols, contamination process and the condition of the victim) and the effectiveness of <u>disinfection techniques</u> (temperature disinfection like pasteurization and shock disinfection, UV radiation, chemical treatment like the addition of free chlorine and copper-silver ionization), while keeping an equilibrium between energy efficient and healthy buildings.

PRACTICAL IMPLICATIONS
Based on the key parameters coming from this literature review it is possible in future research to develop a simulation model that allows to investigate the infection risk for *Legionella pneumophila* in the design phase of a DHW system and to test the effectiveness of disinfection techniques on an infected system. By developing a simulation model that allows assessing the *Legionella pneumophila* infection risk in dynamic conditions, HVAC designers will be able firstly to thoroughly assess the infection risk associated with their design and secondly to optimize the temperature regimes, choose better hydronic controls and reduce the energy demand for DHW production.

KEYWORDS
Legionella pneumophila growth, infection process, water treatment techniques, domestic hot water system

1 INTRODUCTION

Background
In 1976 the first literature about *Legionella* appeared concerning the first documented outbreak in Philadelphia. Over the preceding two decades less research had been published on the progress in knowledge on the infection process and disinfection techniques. Brundrett gave a complete overview in 1992 in *Legionella and Building Services*, hereafter very few updated overview works have been published on this topic (Brundrett, 1992).

Legionnaires' disease is named after the first identified outbreak at the American Legion's Convention at the Bellevue Stratford Hotel in Philadelphia in July 1976. 221 American Legion

Members were struck of which 34 died. It wasn't the first outbreak. It is known from stored tissue that this bacterium was responsible for mystery illnesses 50 years ago. Prior to 1976 there had been an outbreak in the St. Elizabeth's Hospital in Washington DC where 81 patients became ill of which 14 died. Another outbreak was in 1974 at an Oddfellows Convention in the Bellevue-Stratford Hotel in Philadelphia where 20 attendees became sick of which 2 died.

There are 39 species of *Legionella* identified. The most common *Legionella* species is *Legionella pneumophila*. The species is able to induce two kinds of illness which appear 2-10 days after exposure. One is Legionnaires' disease, a life threatening pneumonia of which the victim requires urgent medication. The other disease caused by the species is Pontiac Fever, named after an outbreak in Pontiac, Michigan in 1968. This variant is flue like, non-pneumonic and non-fatal. It wasn't until *Legionella* was discovered after the 1976 outbreak in Philadelphia that public health officials were able to ascertain that the same bacterium caused this disease.

Objectives

The production of Domestic Hot Water (DHW) dominates the total energy demand in well insulated buildings. One of the main reasons for the high energy demand is that DHW is produced, stored and distributed at temperatures above 55°C to mitigate the risk of infecting the DHW system with *L. pneumophila*. *L. pneumophila* is an aerobic gram-negative bacterium that, upon exposure, causes acute respiratory disease (Pontiac Fever) or severe pneumonia (Legionnaires disease). At these temperatures, *L. pneumophila* species are effectively killed. For most of the applications of DHW, temperatures of only 30-40°C are required. This disparity (between 55°C and 30-40°C) doubles the temperature difference between the DHW system and the environment and has a detrimental effect on the efficiency of DHW production units. The overall aim of the research is to lower the energy demand for domestic hot water whilst obtaining a low infection risk. To maintain this objective it is necessary to better understand the principles of infection process and disinfection techniques, this is the object of this paper (Ballanco, 2012; Brundrett, 2003).

2 METHODS - OBJECT OF LITERATURE REVIEW

The <u>growth of *L. pneumophila*</u> is influenced for example by lukewarm water between 20 and 45°C, stagnation, an acid environment, the presence of nutrients and the presence of metals like Fe and Zn.

The <u>infection process</u> consists of four critical steps, all four conditions need to be fulfilled to create a severe risk of illness and death of people exposed to the bacteria. First the bacteria needs profitable conditions to reach a dangerous concentration. The second critical step is the creation of aerosols. These small droplets produced by showers float in air. The third step in the contamination process occurs when the aerosols reach the smallest parts of the lungs; the alveoli. From here the bacteria will reach the bloodstream. The fourth step is the condition of the victim, persons who have a reduced resistance against illnesses are more susceptible, as well as older people, men and smokers.

<u>Water treatment techniques</u> are preventing the amplification of *L. pneumophila* in the installation. The most common method in residential buildings is pasteurization. The temperature in the whole installation is kept above 55-60°C to prevent profitable situations for *L. pneumophila*. Another technique is shock disinfection. During a short period of time, the whole installation is brought to 65-70°C to kill all species. Between these actions the temperature is lowered to be more energy efficient. Less used alternatives like UV radiation, chemical treatment such as the addition of biocides and copper-silver ionization are compared.

This paper offers an overview of the profitable conditions for *L. pneumophila* growth and the effectiveness of disinfection techniques while keeping an equilibrium between energy efficient and healthy buildings.

3 RESULTS

Literature review - Profitable conditions for *Legionella pneumophila* growth

Legionella exists as part of the natural microbial flora of many aquatic ecosystems. *L. pneumophila* species appear in most water supplies like lakes, ponds and rivers, this is harmless, but very low concentrations of *Legionella* from natural habitats can be increased markedly in man-made hot water systems where the temperature is optimal for their growth (Figure 1) (Katz and Hammel, 1987).

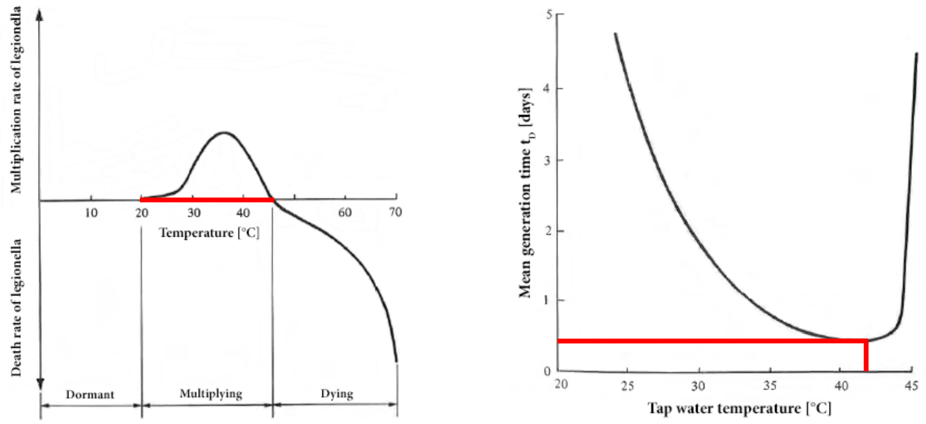

Figure 1. *L. pneumophila* growth curve as a function of temperature (Brundrett, 1992).

L. pneumophila species appear in water and in biofilm. This biofilm structure is composed of a consortium of microbial cells that are attached to the surface and associated together in an extracellular anionic polymer matrix (Donlan, 2002). The matrix is extremely hydrated (97% water) and consists mainly of exopolysaccharides, biological macromolecules (proteins, lipids, DNA and RNA), nutrients, metabolites, and inorganic compounds and particles, as well as cellular lysis products (Farhat et al., 2012). The bacteria attach to the biofilm because it consists of microorganisms which allow cells to adhere, it forms a protective layer for the bacteria which allows them to grow and multiply in the biofilm. Biofilms adjust to their surroundings and can resist antimicrobial agents. According to Flemming et al. (2002), 95% of *Legionella* and other micro-organisms are surface-associated (biofilm).

In the first place you should consider avoiding *L. pneumophila* by regarding some plumbing practices, like avoiding stagnant sections of piping that would allow the growth of biofilm. Stagnation can be prevented by reducing the volumes of stored water and introducing routine flushing programmes.

Studies showed that there were greater microbial levels in water where *L. pneumophila* was detected (determined by Heterotrophic Plate Count (HPC)). The results show that *L. pneumophila* was largely isolated when the HPC mean was between 5 and 6 logs cfu/100mL, which indicated that the presence of *L. pneumophila* species was associated with the presence of biofilm on which it can grow in the hot water pipes. The presence of bacteria is essential for the survival of *L. pneumophila* in water systems, but their presence alone does not determine the occurrence of the pathogen (Temmerman et al., 2006; Serrano-Suárez et al., 2013).

Protozoa were observed in 45% of the hot water samples contaminated with *L. pneumophila*, and amoebas were present in nearly all of them. The presence of protozoa in samples from hot water recirculation systems with storage tanks in hotels and nursing homes was higher when *L. pneumophila* species were present, 53 versus 23%. The regression analysis showed that the values of microbiota and protozoa increased the risk of *L. pneumophila* colonization. Protozoa are protecting the *L. pneumophila* species from harsh conditions. Probably these microorganisms were structured in biofilms from where the *L. pneumophila* species were detached contaminating running water (Serrano-Suárez et al., 2013).

The presence of metals such as Fe or Zn derived from pipelines and fittings are important parameters for bacterial growth and virulence (Reeves et al., 1981; States et al., 1985; Yaradou et al., 2007). Fe favored bacterial growth. *L. pneumophila* species cannot grow in culture media without Fe. The logistic analysis showed that the presence of Fe above 0.095 ppm is associated with *L. pneumophila*. Authors, such as Rogers et al. (1994) and Borella et al. (2004), stated that Cu inhibits its growth. The risk of *L. pneumophila* colonization in the circuits significantly decreased with respect to Cu concentration (detection limit of 0.01ppm) (Serrano-Suárez et al., 2013).

Literature review - Infection process

The infection route consists of four critical steps, all four conditions need to be fulfilled to create a severe risk of illness and death of people exposed to *L. pneumophila* species.

First the *L. pneumophila* species need profitable conditions to multiply and reach a dangerous concentration. The growth of *L. pneumophila* is influenced for example by lukewarm water between 20 and 45°C (Figure 1), stagnation, an acid environment and the presence of nutrients through for example dirt and traces of rust.

The second critical step to get a *L. pneumophila* infection is inhaling the *L. pneumophila* species, therefore the *L. pneumophila* species must become an aerosol. These small droplets float in air. Aerosols are formed by showers, aerated faucets, misters, humidifiers, whirlpool bathtubs, vegetable sprayers, handheld sprayers and water features. It is recommended to use laminar flow aerators or no aerators on faucets in for example hospitals.

The third step in the contamination process occurs when the contaminated aerosols reach the smallest parts of the lungs; the alveoli (Figure 2). From here the bacteria will reach the bloodstream.

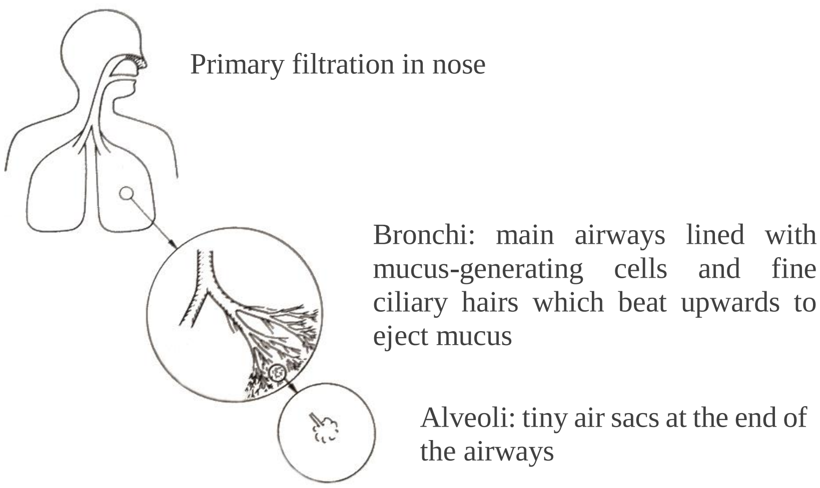

Figure 2. Illustrative guide to the airways of the lung (Brundrett, 1992).

The fourth step is the condition of the victim, persons who have a reduced resistance against illnesses are more susceptible, as well as very young children, older people, men and smokers. The *L. pneumophila* species are known to have greater effects on people with age above 50 years. Males are thrice as likely to catch the disease. It is thought that this may be a result of typical occupations, lifestyles and possibly lungs size. The chances of infection are higher for people with lung damage or another illness. Legionnaires disease is a common hospital associated infection (HAI) because patient immune system is compromised. Many incidents go unreported because hospitals don't look for Legionnaires' disease often with HAI.

Literature review - Water treatment techniques

In this paper, a review of water disinfection techniques is provided since the effectiveness of all treatments on *L. pneumophila* in water and in biofilm must be made explicit in order to allow a reliable performance assessment. A broader review of literature on the results of disinfection studies is offered.

Killing *L. pneumophila* can be done by hot temperature, ultraviolet radiation, chemical treatment like hyper chlorination or addition of chlorine dioxide and copper-silver ionization. Several authors have reported that bacteria present in biofilm become more resistant to environmental stress. By adoption of life in biofilm, *Legionellae*, like other bacteria, are far less susceptible to any kind of stress coming from the outside. In addition, these bacteria survive as an intracellular parasite of free-living amoebae (Farhat et al., 2012). Free-living amoebae are eukaryotic microorganisms, commonly found in drinking water systems, with phagocyte bacteria as their nutritional source. Rowbotham (2015) described for the first time in 1980 that *L. pneumophila* not only survives digestion by amoeba but also use the amoeba host nutritional sources to replicate intracellularly. This intracellular state also protects *Legionella* against environmental factors and water disinfection treatments (Cervero-Aragó et al., 2015).

<u>Thermal treatments</u> and chlorine are the most commonly used procedures worldwide to control and prevent *Legionella* proliferation in drinking water systems of large buildings. In case of thermal treatments, as suggested by the World Health Organization (WHO), water flow temperature should be kept at a minimum of 60°C when leaving the heating unit and at least 50°C when it reaches the tap (Cervero-Aragó et al., 2015).

Farhat et al. stated in 2010 that thermal disinfection does not seem to be efficient enough to eliminate *Legionella* when it is used as a curative treatment. They did measurements on a test loop starting from a stable cultivable *Legionella* spp. concentration of 5*10^5cfu/l. Two heat shock treatments of 70°C for the duration of 30min were applied. The results showed that the first treatment had a transitional effect on the abatement of *Legionella* concentrations, while the second treatment had no detectable effect on *Legionella* populations in water and biofilm. A resistance test was conducted and showed that *Legionella* in the second heat shock treatment was not thermo-resistant but thermo-acclimated. The concentration of *Legionella* (PCR) in the dead leg water of the test loop was 2 log units higher than in the test loop water. These analyses revealed that they are responsible for the rapid recolonization of the water in the circuit as well as in the incompletely destroyed biofilm.

<u>Ultraviolet radiation</u> is good at controlling *L. pneumophila* growth, although a long dwell time may be required. Disadvantages are that the maintenance of the system is high and a filter is required ahead of the UV unit.

Free chlorine is mostly used at a low concentration (0.2-0.5mg/l) as a secondary disinfectant for the maintenance of water quality in distribution systems or at higher concentrations as an installation disinfection treatment called hyper chlorination (Cervero-Aragó et al., 2015). Chlorination has a lot of disadvantages. 4 tot 6ppm of chlorine only provides 90% kill of *L. pneumophila* species. Drinking water contains approximately 0.75ppm of chlorine. Chlorine dioxide can damage the plumbing components.

Copper-silver ionization is one of the most effective means of killing *L. pneumophila* species. The system injects small quantities of copper and silver into the water.

Effect of water treatment techniques on *Legionella pneumophila* in biofilm

Cervero-Aragó et al. (2015) tested the effect of temperature and free chlorine applied in similar exposure conditions as in drinking water systems on *L. pneumophila* strains and two amoebae strains under controlled laboratory conditions. To determine the influence of the relationship between *L. pneumophila* and amoebas *Acanthamoeba spp.* and *A. Castellani* on the treatment effectiveness, inactivation models of the bacteria-associated amoeba were constructed and compared to the models obtained for the free living bacteria state.

Several free chlorine concentrations were tested in co-culture experiments: 0.5mg/l, 1.2mg/l and 2.5mg/l. The inactivation kinetics were adjusted to first-order models. R^2 values show the robustness of the models (Table 1).

Table 1. Calculation time for a 4-log reduction of *L. pneumophila* sg. 1 env. associated with *A. Castellani* CCAP 1534/2 and *Acanthamoeba* sp. 155 after the exposure to different concentrations of free chlorine.

Calculated time (min) to reduce 4 logs						
The disinfection effect of free chlorine treatment on free *Legionella*	**0.5mg/l (R^2)**		**1.2mg/l (R^2)**		**2.5mg/l (R^2)**	
L. pneumophila sg. 1 env (Axenic)	5	(0.96)	-	(-)	-	(-)
The effect of chlorine on amoebae-associated *Legionella*	**0.5mg/l (R^2)**		**1.2mg/l (R^2)**		**2.5mg/l (R^2)**	
L. pneumophila sg. 1 env - *A. Castellanii* CCAP 1534/2	490	(0.85)	152	(0.76)	43	(0.79)
L. pneumophila sg. 1 env - *Acanthamoeba* sp. 155	38	(0.54)	17	(0.64)	23	(0.82)

The results show significant differences ($p<0.001$) between the inactivation of the axenic *L. pneumophila* and *L. pneumophila* associated with protozoa. Axenic *L. pneumophila* reached a 4-log reduction after 5min at 0.5mg/l, whereas *L. pneumophila* associated with *Acanthamoeba* sp. 155 required 38min and *L. pneumophila* associated with *A. Castellani* CCAP 1534/2 required 490min to reach such a reduction. Remarkably, at the lowest free chlorine concentration, 0.5mg/l, the influence of the *Legionella*-amoeba associate state was the strongest in reducing the effectiveness of the treatments compared to the free *Legionella* state (Cervero-Aragó et al., 2015).

Other researchers also reported a higher resistance of *L. pneumophila* to chlorine when it lived intracellularly within *Acanthamoeba* strains. The *Legionella*-amoeba association did not change the inactivation models, but it reduced the effectiveness of the treatments applied. The failure of disinfectants in controlling *Legionella* in domestic hot water systems has been attributed to the presence of protozoan hosts that act as a shield for pathogenic bacteria against disinfectants (Cervero-Aragó et al., 2015).

A thermal treatment at four experimental temperatures was tested: 50°C, 55°C, 60°C and 70°C, for various exposure times and applied to *Legionella* spp. strains under controlled laboratory conditions.

Table 2. Calculation time for a 4-log reduction of *L. pneumophila* sg. 1 env. associated with *A. Castellani* CCAP 1534/2 and *Acanthamoeba* sp. 155 after the exposure to different temperatures.

Calculated time (min) to reduce 4 logs				
The effect of temperature on free *Legionella*	**50°C (R^2)**	**55°C (R^2)**	**60°C (R^2)**	**70°C (R^2)**
L. pneumophila sg. 1 env (Axenic)	46 (0.84)	8 (0.98)	4 (0.86)	0.61 (0.82)
The effect of temperature on amoebae-associated *Legionella*	**50°C (R^2)**	**55°C (R^2)**	**60°C (R^2)**	**70°C (R^2)**
L. pneumophila sg. 1 env - *A. Castellanii* CCAP 1534/2	825 (0.56)	45 (0.84)	5 (0.99)	0.45 (0.82)
L. pneumophila sg. 1 env - *Acanthamoeba* sp. 155	664 (0.95)	51 (0.95)	5 (0.73)	0.50 (0.92)

The time required for the cultivability of *L. pneumophila* to reach a 4-log reduction for the axenic *L. pneumophila* sg. 1 was 46min at 50°C, 8min at 55°C, 4min at 60°C and 0.61min at 70°C (Table 2). The effect of thermal treatments on *L. pneumophila* associated with *Acanthamoeba* strains fits a first-order (straight line) model. When *L. pneumophila* associated with either *Acanthamoeba* strains or *A. Castellani* strains, these times ranged from 664-825min at 50°C, 51-45min at 55°C, 5-5min at 60°C and 0.50-0.45min at 70°C, respectively.

The effectiveness of the thermal treatment compared to the free form was reduced. At 50°C, the bacterial resistance was increased between 14-18 fold, and at 55°C it was increased between 5 and 6 fold. Thus, it seems that *Acanthamoeba* and *A. Castellani* strains play a protective role for the bacteria at temperatures below 60°C, but at higher temperatures, its protection dramatically decreases (Cervero-Aragó et al., 2015).

4 DISCUSSION

Over the preceding two decades less research had been published on the advancement in knowledge on the *Legionella pneumophila* infection process and disinfection techniques. Brundrett gave a complete overview in 1992, hereafter very few updated overview works have been published on this topic. This paper gives a review of the work done on this topic between 1992 and 2016 by stating the most important research findings.

This paper offers an overview of the profitable conditions for *Legionella pneumophila* growth. Profitable conditions are lukewarm water between 20 and 45°C, stagnation, an acid environment, the presence of nutrients and metals such as Fe and Zn. The infection process consists of four steps; the *L. pneumophila* species need profitable conditions to multiply, the next step is the creation of aerosols, the contamination process occurs when the contaminated aerosols reach the smallest parts of the lungs and the last important parameter is the condition of the victim. The effectiveness of disinfection techniques like pasteurization, shock disinfection, UV radiation, chemical treatment and copper-silver ionization are researched. The influence of the *Legionella*-amoeba associate state is investigated for free chlorine and temperature disinfection. The effectiveness of thermal treatments applied increased as the temperatures and exposure times increased, especially for temperatures higher than 55°C. Similar to the free chlorine concentrations, it can be noticed that at the lowest temperatures, 50°C and 55°C, the influence of the *Legionella*-amoeba associate state was the strongest in reducing the effectiveness of the treatments compared to the free *Legionella* state. Therefore, the association established between *L. pneumophila* and amoebae in domestic hot water systems indicate an increased health risk (Cervero-Aragó et al., 2015).

There is still a large gap of knowledge in literature about the influence of disinfection techniques on the growth of *Legionella pneumophila* species. More research is needed on this subject to achieve energy efficient and healthy buildings.

5 CONCLUSIONS

This research made it possible to better understand the principles and define the important parameters for *Legionella pneumophila* growth, the infection process and the disinfection techniques. Based on the key parameters coming from this literature review it is possible to develop a simulation model that allows to investigate the infection risk for *Legionella pneumophila* in the design phase of a DHW system and to test the effectiveness of disinfection techniques on an infected system. By developing a simulation model that allows assessing the *Legionella pneumophila* infection risk in dynamic conditions, HVAC designers will be able firstly to thoroughly assess the infection risk associated with their design and secondly to optimize the temperature regimes, choose better hydronic controls and reduce the energy demand for DHW production.

ACKNOWLEDGEMENT

This research is founded by the Agency for Innovation by Science and Technology-Belgium (IWT), Project 141608. The authors thank the Belgian Building Research Institute (BBRI/WTCB/CSTC) and especially Karla Dinne and Bart Bleys for making it possible to build the test rig and making their experimental data available. The authors thank the partners of the Proeftuin R&D building projects.

6 REFERENCES

Ballanco J. 2012. Controlling *Legionella* in Plumbing Systems, presentation.

Borella P., Montagna M.T. et al. 2004. *Legionella* infection risk from domestic hot water. *Emerging Infectious Diseases*, 10(3), 457-464.

Brundrett G. 1992. *Legionella and Building Services*. Oxford.

Brundrett G.W. 2003. Preventing Legionellosis: is your action plan completed?. Facilities, 21(11/12), 275-279.

Cervero-Aragó S., Rodríguez-Martínez, S. et al. 2015. Effect of common drinking water disinfectants, chlorine and heat, on free *Legionella* and amoebae-associated *Legionella*. *PLoS ONE,* 10(8).

Donlan R.M. 2002. Biofilms: microbial life on surfaces. *Emerging Infectious Diseases*, 8(9), 881-890.

Farhat M., Moletta-Denat M. et al. 2012. Effect of disinfection on *Legionella* spp., Eukarya, and biofilms in a hot water system. *Applied and Environmental Microbiology*, 78(19), 6850-6858.

Farhat M., Trouilhe M.-C. et al. 2010. Development of a pilot-scale 1 for *Legionella* elimination in biofilm in hot water network: heat shock treatment evaluation. *Journal of Applied Microbiology*, 108(3), 1073-1082.

Flemming H.C., Walker J.T. 2002. Contamination potential of biofilms in water distribution systems. *Water Science and Technology: Water Supply*, 2(1), 271-280.

Katz S.M. and Hammel J.M. 1987. The effect of drying, heat, and PH on the survival of *Legionella pneumophila*. *Annals of clinical and laboratory science*, 17(3), 150-156.

Reeves M.W., Pine L. et al. 1981. Metal requirements of *Legionella-pneumophila*. *Journal of Clinical Microbiology*, 13(4), 688-695.

Rogers J., Dowsett A.B. et al. 1994. Influence of plumbing materials on biofilm formation and growth of *Legionella pneumophila* in potable water systems. *Appl. Environm. Microbiol.*, 60(6), 1842-1851.

Rowbotham T.J. 1980. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. *J. Clin. Path.*, 33, 1179-1183.

Serrano-Suárez A., Dellundé J. et al. 2013. Microbial and physicochemical parameters associated with *Legionella* contamination in hot water recirculation systems, *Environmental Science and Pollution Research*, 20(8), 5534-5544.

States S.J., Conley L.F. et al. 1985. Effects of metals on *Legionella pneumophila* growth in drinking water plumbing systems. *Applied and Environmental Microbiology*, 50(5), 1149-1154.

Temmerman R., Vervaeren H. et al. 2006. Necrotrophic growth of *Legionella pneumophila*. *Applied and Environmental Microbiology*, 72(6), 4323-4328.

Yaradou D.F., Raze D. et al. 2007. Zincdependent cytoadherence of *Legionella pneumophila* to human alveolar epithelial cells in vitro. *Microb. Pathog.*, 43, 234-242.